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Susceptible and Protective Human Leukocyte Antigen Class II Alleles and Haplotypes in Bahraini Type 2 (Non-Insulin-Dependent) Diabetes Mellitus Patients

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Whereas the genetic risk for type 1 diabetes is linked to human leukocyte antigen (HLA) class II genes, the HLA association in type 2 (non-insulin-dependent) diabetes is less clear. The association between HLA class II genotypes and type 2 diabetes was examined in adult Bahrainis, an Arab population with a high prevalence of type 2 diabetes. HLA-DRB1* and -DQB1* genotyping of 86 unrelated type 2 diabetes patients (age, 51.6 ± 8.2 years; mean duration of diabetes, 7.7 ± 7.1 years) who had a strong family history of diabetes (52 of 72 versus 0 of 89 for controls, $P < 0.001$) and 89 healthy subjects was done by PCR-sequence-specific priming. DRB1*040101 (0.1221 versus 0.0562, $P = 0.019$) and DRB1*070101 (0.2151 versus 0.0843, $P < 0.001$) were positively associated, while DRB1*110101 (0.0698 versus 0.1461, $P = 0.014$) and DRB1*160101 (0.0640 versus 0.1236, $P = 0.038$) were negatively associated with type 2 diabetes. DRB1*040101-DQB1*0302 (0.069 versus 0.0007; $P = 0.004$), DRB1*070101-DQB1*0201 (0.178 versus 0.0761, $P = 0.007$), DRB1*070101-DQB1*050101 (0.125 versus 0.0310, $P = 0.002$), and DRB1*150101-DQB1*060101 (0.0756 versus 0.0281, $P = 0.008$) were more prevalent among patients, while DRB1*160101-DQB1*050101 (0.0702 versus 0.0349, $P = 0.05$) was more prevalent among controls, conferring disease susceptibility or protection, respectively. In Bahrainis with type 2 diabetes, there is a significant association with select HLA class II genotypes, which were distinct from those in type 1 diabetes.

Whereas type 2 (non-insulin-dependent) diabetes mellitus is the most common form of diabetes, its specific etiology is not yet known (31, 34). Its frequency varies in different racial and ethnic subgroups (31, 34) and is often associated with a strong familial, likely genetic, predisposition (31, 34), more so than autoimmune type 1 (insulin-dependent) diabetes mellitus (31). The genetics of type 2 diabetes are complex and not clearly defined (31, 34). Among a subgroup of autoimmune type 1 diabetes, latent autoimmune diabetes in adults, there is a slowly progressive form of β cell destruction generally occurring in adults who present with an initial clinical picture of type 2 diabetes but who have autoantibodies found in type 1 diabetes and who subsequently develop insulin deficiency (34).

While the role for HLA in the pathogenesis of type 1 diabetes was reported by several groups (11, 17), its role in type 2 diabetes is less clear, and weak links between human leukocyte antigen (HLA) class II and type 2 diabetes were reported for some ethnic groups. Previous investigation of the contribution of HLA class II in type 2 diabetes pathogenesis focused on HLA relationship with autoimmune markers and latent autoimmune diabetes in adults (4, 15, 32), possible genetic interaction between type 1 and type 2 diabetes in families (20, 21, 26), and its association with complications and mortality in type 2 diabetes patients (6, 13, 25).

Very few studies examined the prevalence of HLA class II allele and haplotype per se, and most examined a possible role for HLA class II in type 2 diabetes in relation to autoimmune markers and latent autoimmune diabetes in adults, genetic interaction between type 1 diabetes and type 2 diabetes, and association with complications of diabetes. This was exemplified by the findings that DRB1*0405 (7), DQB1*0201 and DQB1*0302 (32), DRB1*03/04-DQB1*0302 (15), and DRB1*03-DQA1*0502-DQB1*0201 (DR3-DQ2) (8) were positively associated with type 2 diabetes in anti-glutamic acid decarboxylase (GAD)-positive type 2 diabetes subjects. In addition, increased frequency of HLA-DR3 and -DR4 was reported in islet cell autoantibody-positive patients with secondary failure to oral antidiabetic drugs (10, 12). Increased frequency of HLA-DRB1*1502 was also seen in anti-GAD-positive patients who remained well controlled on oral antidiabetic drugs (7). Furthermore, tumor necrosis factor alpha was associated with predisposition to subsequent insulin dependency in GAD-positive HLA-DRB1*1502-DQB1*0602 (22) or DQB1*02 (19) type 2 diabetes patients, suggesting an interplay between HLA genotypes and other polymorphic markers in dictating the progression of type 2 diabetes. Others failed to link HLA class II (DQA1 and DQB1) with type 2 diabetes (4, 5), and it would appear that the association between HLA class II and type 2 diabetes is racially and geographically restricted.

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TABLE 1. Clinical characteristics of study participants[a]

Parameter	Controls	Patients	*P*
No.	89	86	
Gender (M:F)	43:46	41:45	0.498
Mean age ± SD (yr)	49.3 ± 5.9	51.6 ± 8.2	0.063
Age at disease onset (yr)		43.5 ± 11.7	
Duration of disease (yr)		8.5 ± 7.3	<0.001
Therapy			
Oral hypoglycemics		70	
Insulin only		15	
Oral + insulin		4	
No. with family history of diabetes/total	0/89	52/72[b]	<0.001
Body mass index (kg/m^2)	24.32 ± 4.23	29.36 ± 5.80	<0.001
Chemistry			
Fasting plasma glucose (mmol/liter)	5.96 ± 2.83	10.53 ± 3.32	<0.001
Creatinine (μmol/liter)	57.46 ± 13.92	74.38 ± 37.90	0.026
HbA_{1c} (%)	5.24 ± 1.39	8.76 ± 2.21	<0.001

[a] *P* was determined by Pearson's χ^2 test.
[b] Includes patients for whom reliable family history could be obtained.

Recently, we demonstrated in Bahraini type 1 diabetes patients that the genetic risk for disease pathogenesis is strongly linked to HLA class II loci, as select HLA-DRB1 and -DQB1 alleles and haplotypes conferred disease susceptibility or resistance to type 1 diabetes (1). Bahrainis, an Arabian Peninsula population, have previously been reported to have a high prevalence of type 2 diabetes (2), and to date there are no reports on the association of HLA with the pathogenesis of type 2 diabetes. This study was therefore undertaken to examine the association between HLA class II genotypes and type 2 diabetes in this population.

MATERIALS AND METHODS

Subjects. The study group included 86 unrelated adult patients (45 females and 41 males; mean age, 51.6 ± 8.2 years), who were diagnosed as type 2 diabetes based on 1998 World Health Organization diagnostic and classification criteria (34) and who attended the diabetes clinics at two hospitals in Bahrain. None of the patients had ever had ketoacidosis. Treatment for diabetes included diet and/or oral antidiabetic drugs and/or insulin (to achieve glycemic control); all subjects commenced on insulin therapy had been treated with oral drugs for at least 2 years (Table 1). Family history of diabetes (52 of 72) or body mass index did not influence selection of subjects.

The control group included 89 unrelated healthy subjects (46 females and 43 males; mean age, 49.3 ± 5.9 years) with no known personal or family history of diabetes. None were first-degree relatives of other subjects in the control group or study group; they were not known to have diabetes, although occult disease was not excluded. All participants were Bahraini Arabs, and non-Arab Bahrainis or recently naturalized Bahrainis were excluded. The Arabian Gulf University Ethics Committee approved the study (which was done according to Helsinki guidelines), and informed consent was obtained from all participants according to the study protocol.

For all subjects, demographic details were recorded, which included age; gender; ethnic origin; age of onset, duration, and first-degree family history of diabetes; history of hypertension, dyslipidemia, ischemic heart disease, and other medical illness; history of chronic complications of diabetes, treatment for diabetes, including date of initiation and/or discontinuation of oral agents or insulin; and history of other medication. Where available, the historical information was verified from the clinic records. After an overnight fast, venous blood samples were collected for measurement of plasma venous glucose, autoantibodies, HLA genotype, HbA_{1c}, serum urea, and electrolytes and liver function tests; albumin-creatinine ratio was measured on a spot urine sample.

Autoantibody measurements. Anti-GAD, islet cell autoantibody, and insulin autoantibodies were determined by enzyme immunoassay on two separate occasions independently, with the Biomerica (Newport Beach, Calif.) and DRG International (Marburg, Germany) assay kits. Each sample was tested twice on each kit system, and results were scored as positive or negative, as per manufacturer's specification.

HLA genotyping. Total genomic DNA was extracted from the peripheral blood mononuclear leukocytes from study participants by the phenol-chloroform method, as is standard, and was used for PCR analysis. The HLA-DRB1 and–DQB1 gene alleles were analyzed with the PCR sequence-specific priming technique, with the SSP2L HLA class II (DRB/DQB) genotyping kit according to the manufacturer's specifications (One Lambda, Thousand Oaks, Calif.). PCR products were analyzed on 2.5% (wt/vol) agarose gels stained with ethidium bromide (0.5 μg/ml).

Data analysis. Allele frequencies were determined as described previously (11) with the HLAStat 2000 software, which also computed the *P* values and odds ratios. The frequencies of the most frequent haplotypes were determined by the maximum likelihood method, with the Arlequin population genetics data analysis software (28). Additional statistical analysis was performed with SPSS version 12.0 for Windows statistical package.

RESULTS

Clinical characteristics. The study group included 86 type 2 diabetes subjects and the control group comprised 89 subjects, who were matched for gender (41 males and 45 females versus 43 males and 46 females, $P = 0.498$) and age (51.6 ± 8.2 versus 49.3 ± 5.9 years, $P = 0.063$). In the study group, the mean age at diagnosis of diabetes was 43.5 ± 11.7 years, and mean duration of diabetes (at entry into the study) was 8.5 ± 7.3 years (Table 1). When compared with the control group, the study subjects had a higher body mass index (29.36 ± 5.80 versus 24.32 ± 4.23 kg, $P < 0.001$) and higher mean fasting plasma glucose ($P < 0.001$) and HbA_{1c} ($P < 0.001$). There was a low prevalence of anti-GAD ($P = 0.0637$), islet cell autoantibody ($P = 0.05$), insulin autoantibodies ($P = 0.188$), and the "double-positive" islet cell autoantibody-insulin autoantibodies ($P = 0.138$) in the study and control groups, which were not different between the groups (Table 1). In addition, there was a strong family history of diabetes among patients (52 of 72) but not among controls (0 of 89) ($P < 0.001$).

Frequencies of HLA-DRB1 and -DQB1 alleles. The distribution of the HLA-DRB1 alleles is summarized in Table 2. The allelic frequencies of DRB1*040101 and DRB1*070101 were significantly higher in the study group than in the control group (Table 2). The frequency of DRB1*110101, DRB1*160101, and to a lesser extent DRB1*1413 was lower in type 2 diabetes patients (Table 2). Differences in the frequencies of other DRB1 alleles were not different between the study and control groups. In contrast to DRB1 alleles, no differences in the frequencies of DQB1 alleles were observed between the two groups (Table 3).

DRB1-DQB1 haplotype frequency. Table 4 shows the distribution of the HLA-DRB1 and -DQB1 haplotypes. The haplotypes which were positively associated with type 2 diabetes included, in order of strength, DRB1*040101-DQB1*0302 ($P = 0.004$, RR 13.28); DRB1* 070101-DQB1*050101 ($P = 0.002$, RR 4.20); DRB1* 150101-DQB1*060101 ($P = 0.008$, RR 2.83); and DRB1*070101-DQB1*0201 ($P = 0.002$, RR = 2.58). A negative association was observed for DRB1*160101-DQB1*050101 ($P = 0.05$, RR = 0.48). No relationship was observed for any specific genotype or combinations in subjects who were autoantibody positive (data not shown).

HLA class II haplotypes in Bahraini type 1 and type 2 diabetes patients. We compared the HLA class II haplotypes associated with type 2 diabetes with the haplotypes associated

TABLE 2. HLA-DRB1 alleles in patients and control subjects[a]

DRB1 allele	Patients		Controls		χ^2	P	R.R.
	Frequency	SE	Frequency	SE			
010101	0.0291	0.0128	0.0169	0.0096	0.599	0.439	1.668
030101	0.0988	0.0228	0.1461	0.0265	1.389	0.239	0.650
030201	0.0233	0.0115	0.0056	0.0056	1.961	0.161	3.218
0310	0.0000	0.0000	0.0169	0.0096	2.949	0.086	0.143
0317	0.0000	0.0000	0.0056	0.0056	0.000	1.000	0.341
040101	0.1221	0.0250	0.0562	0.0173	5.465	0.019	2.612
0412	0.0000	0.0000	0.0056	0.0056	0.000	1.000	0.341
070101	0.2151	0.0313	0.0843	0.0208	11.414	<0.001	3.260
080101	0.0174	0.0100	0.0281	0.0124	0.455	0.500	0.644
080201	0.0174	0.0100	0.0112	0.0079	0.243	0.622	1.467
090102	0.0116	0.0082	0.0112	0.0079	0.0012	0.972	1.036
100101	0.0523	0.0170	0.0730	0.0195	1.165	0.280	0.614
110101	0.0698	0.0194	0.1461	0.0265	5.991	0.014	0.402
1117	0.0058	0.0058	0.0000	0.0000	0.000	1.000	3.140
120101	0.0233	0.0115	0.0169	0.0096	0.187	0.666	1.348
130101	0.0233	0.0115	0.0225	0.0111	0.002	0.960	1.036
130701	0.0116	0.0082	0.0449	0.0155	3.604	0.058	0.284
1324	0.0058	0.0058	0.0000	0.0000	0.000	1.000	3.140
1327	0.0058	0.0058	0.0281	0.0124	2.622	0.105	0.270
140101	0.0291	0.0128	0.0393	0.0146	0.288	0.591	0.742
1411	0.0058	0.0058	0.0056	0.0056	<0.001	0.981	1.035
1413	0.0000	0.0000	0.0337	0.0135	3.956	0.047	0.110
150101	0.1570	0.0277	0.0730	0.0195	2.690	0.101	1.860
160101	0.0698	0.0194	0.1461	0.0265	4.298	0.038	0.437

[a] DRB1∗ alleles were assessed by PCR-SSP. A total of 86 patients and 89 control subjects were analyzed. Allele frequency was determined as number of an allele ÷ total number of alleles per group. *P* was determined by Fisher's exact test.

with type 1 diabetes among Bahraini patients. DRB1*030101-DQB1*0201 and DRB1* 030101-DQB1*0302, while conferring disease susceptibility in type 1 diabetes, were neutral in type 2 diabetes (Table 5). Similarly, DRB1* 070101-DQB1*050101, DRB1* 040101-DQB1*0302, and DRB1* 150101-DQB1*060101, while positively associated with type 2 diabetes, were not associated with type 1 diabetes (Table 5). Furthermore, DRB1* 070101-DQB1*0201 was positively associated (susceptible) with type 2 diabetes, but negatively associated (protective of) with type 1 diabetes (Table 5). Collectively, this demonstrated that the HLA class II usage in type 2 diabetes is distinct from that of type 1 diabetes among Bahraini patients.

TABLE 3. HLA-DQB1 alleles in patients and control subjects[a]

DQB1 allele	Patients		Controls		χ^2	P	R.R.
	Frequency	SE	Frequency	SE			
0201	0.3023	0.0350	0.2472	0.0323	0.932	0.334	1.337
030101	0.1279	0.0255	0.1629	0.0277	0.491	0.484	0.793
0302	0.1105	0.0239	0.0899	0.0214	0.475	0.491	1.298
03032	0.0349	0.0140	0.0449	0.0155	0.050	0.823	0.888
0401	0.0407	0.0151	0.0337	0.0135	0.004	0.951	1.037
050101	0.2267	0.0319	0.3034	0.0345	2.576	0.108	0.617
060101	0.1570	0.0277	0.1180	0.0242	0.698	0.403	1.338

[a] See Table 2, footnote *a*.

TABLE 4. DRB1∗-DQB1∗ haplotype distribution among patients and controls[a]

DRB1/DQB1	Frequency		*P*	χ^2	R. R.	95% C.I.
	Patients	Controls				
030101/0201	0.0871	0.1299	0.28	1.19	0.64	0.33–1.28
040101/0302	0.0698	0.0007	0.004	8.35	13.28	1.67–50.80
070101/0201	0.178	0.0761	0.007	7.18	2.58	1.30–4.89
070101/050101	0.125	0.0310	0.002	9.31	4.20	1.61–9.76
0801/03011	0.0174	0.0147	0.71	0.14	1.04	0.23–4.62
1001/0501	0.0485	0.0660	0.54	0.38	0.68	0.28–1.69
110101/030101	0.0509	0.1077	0.09	2.82	0.46	0.21–1.06
120101/030101	0.0006	0.0169	0.64	0.22	0.34	0.06–3.00
130101/060101	0.0170	0.0165	0.71	0.14	1.04	0.23–4.62
150101/060101	0.0756	0.0281	0.008	7.14	2.83	1.32–5.70
160101/050101	0.0349	0.0702	0.05	3.69	0.48	0.24–0.98

[a] DRB1∗ and DQB1∗ alleles were assessed by PCR-SSP, and haplotype frequencies were determined by the maximum-likelihood method. A total of 86 patients and 89 control subjects were analyzed. *P* was determined by Fisher's exact test. C.I., confidence interval.

DISCUSSION

This study has shown that in Bahrainis with type 2 diabetes, there is a significant association with select HLA-DRB1 and -DQB1 alleles and haplotypes; with positive association seen with DRB1*-040101 and −070101 alleles, and DRB1*040101-DQB1*0302, DRB1*070101-DQB1*050101, DRB1*150101-DQB1*060101, and DRB1*070101-DQB1*0201 haplotypes. Moreover, negative association was seen with the DRB1*-110101 and −160101 alleles, and the DRB1*160101-DQB1*050101 haplotype. While association of DRB1*070101 was reported for Mexican (24) and Turkish (3) type 2 diabetes patients, no other study has reported a positive association between type 2 diabetes and these genotypes (7, 9, 12), including studies that used HLA genotyping methods (9, 14).

Earlier studies with serologic markers suggested an association between type 2 diabetes and class I (5, 23, 33) or class III (18) but not class II antigens (5) in non-European populations (16, 23, 29, 30). By contrast, for Europeans most studies suggested that there was no HLA association in type 2 diabetes, with the notable exception of two studies on Finnish subjects in which class I (11, 12) and class II antigens (12) were associated with type 2 diabetes. In the latter study, type 2 diabetes patients had a higher frequency of HLA-B7, -DR2, -DR5, -Cw4,

TABLE 5. HLA class II haplotypes in Bahraini type 1 and type 2 diabetes patients

Haplotype	Effect in patients with:	
	Type 1 diabetes[a]	Type 2 diabetes
DRB1∗100101-DQB1∗050101	Protective	Neutral
DRB1∗030101-DQB1∗0201	Susceptible	Neutral
DRB1∗030101-DQB1∗0302	Susceptible	Neutral
DRB1∗110101-DQB1∗050101	Protective	Neutral
DRB1∗110101-DQB1∗030101	Protective	Neutral
DRB1∗070101/0201	Protective	Susceptible
DRB1∗070101/050101	Neutral	Susceptible
DRB1∗150101-DQB1∗060101	Neutral	Susceptible
DRB1∗040101/0302	Neutral	Susceptible
DRB1∗160101/050101	Neutral	Protective

[a] Data from Al-Harbi et al. (1).

and -DR3/4 when compared with either type 1 diabetes patients or controls (12). Furthermore, in two studies with genotyping methods, no association was found with class II antigens (DR, DQ, and DX) in Punjabi Sikhs (14), while a positive association with HLA-DQA genes was reported for Belgians (9).

In the limited reports in Arab populations with serological HLA typing (16, 27), no association was found for class I or III antigens in Iraqis (16), while in a mixed cohort of Kuwaiti type 1 and type 2 diabetes subjects, HLA-DR3 and -DR4 were present in 50% of subjects, but this was related to younger age at disease onset and a positive family history (27).

The positive association of DRB1*070101 with type 2 diabetes was reminiscent of similar findings for Mexican (24) and Turkish (3) patients, suggesting a role for it in type 2 diabetes pathogenesis. However, the negative association reported here for DRB1*110101 or for DRB1*160101 has not been previously reported for type 2 diabetes in other populations and is probably accounted for by the fact that hardly any studies have examined only the prevalence of the DRB1 alleles with type 2 diabetes.

Although the DQB1 region was examined in some of the studies on the relationship with autoimmune markers (4, 15, 32) and in some family studies (21, 26), direct comparison was not possible, despite the fact that a few studies have examined DQB1*0201 (8, 20, 26, 32) or DQB1*0601 (15, 20, 22). This is so because these studies reported on genotype combinations (see below) and not on the frequency of the specific genotype. While there was no association of a particular DQB1 allele individually with Bahraini type 2 diabetes patients, the presence of specific DQB1 alleles, in particular DQB1*050101 and DQB1*060101, in specific haplotypes conferred disease susceptibility (DRB1*15010101-DQB1*060101) or resistance (DRB1*16010101-DQB1*050101).

The uniqueness of the HLA class II usage in Bahraini type 2 diabetes patients was evidenced by comparing DRB1-DQB1 haplotypes most prevalent in type 1 diabetes and type 2 diabetes patients (Table 5). It was clear that there was distinct association specific of DRB1-DQB1 haplotypes in either diabetes types. For example, "disease-susceptible" haplotypes in type 1 diabetes, such as DRB1*03010101-DQB1*0201 (1), were neutral in type 2 diabetes, while the DRB1*070101-DQB1*050101 "type 2 diabetes-susceptible" haplotype was neutral in type 1 diabetes patients (1). In view of the similar ethnic backgrounds of type 1 and type 2 diabetes patients compared above, this confirms the uniqueness of the HLA class II usage in Bahraini type 2 diabetes patients.

Previous studies reported comparable associations of HLA-DRB/DQB haplotypes in type 2 diabetes, together with "type 1 high-risk" genotypes, including HLA-DQB1*0201/0302 (32) and HLA -DRB1*04-DQB1*0302 (15), both of which were present at higher frequencies in GAD-positive type 2 diabetes patients. By contrast, no association was found with the latter combination in this study, and GAD positivity among Bahraini type 2 diabetes patients (5.81%) was significantly lower than that of type 1 diabetes patients (32.61%) of the same ethnic and socioeconomic background (1). Of interest is that one of the positively associated genotype combinations included HLA-DQB1* 0201, a "high-risk" genotype for type 1 diabetes in Caucasians, especially in association with HLA-DRB1*03 (15).

Based on our earlier and current findings, it is tempting to speculate that depending on the DRB1* genotype they are associated with, DQB1* 0201 and DQB1*050101 may confer "type 1" or "type 2" diabetes susceptibility or resistance. Clearly, this would need to be confirmed in studies in other ethnic groups. Of interest was the increased prevalence of the DRB1*150101-DQB1*060101 haplotype among Bahraini patients with type 2 diabetes. Previous studies which looked into the possible role of DQB1*0601 reported that in GAD-positive, DRB1*1502-QB1*0601-positive patients initially diagnosed as having type 2 diabetes, tumor necrosis factor alpha was associated with predisposition to subsequent insulin dependency (19, 22). In the Finnish study (8), the frequency of the "type I protective alleles" HLA-DQB1*0602 and *0603 was similar in control subjects and GAD-positive and GAD-negative type 2 diabetes patients, but lower in type 1 diabetes subjects.

HLA class II molecules play a decisive role in presenting antigens, including autoantigens, during the T-cell activation cascade. While the implications of our findings remain to be determined, it is tempting to speculate that the positive and negative association of the *DRB1*-DQB1** haplotypes with type 2 diabetes is due to differential affinity for antigenic fragments presented by each haplotype. Accordingly, susceptible haplotypes may bind to and present specific antigens, thereby precipitating hypoglycemia, while "protective" haplotypes may have reduced or no affinity for such antigens.

In conclusion, this study has clearly demonstrated that in Bahrainis, a population with a high prevalence of type 2 diabetes (>22% of the adult population), there is a significant association with both HLA-DRB1 and -DQB1 genotypes, with some alleles and haplotypes appearing to confer susceptibility and others playing a protective role. A larger study which addresses class II genotype distribution among type 2 diabetes from other communities, in particular those of neighboring countries (Qatar, Kuwait, and Oman) with a high prevalence of type 2 diabetes, is needed to confirm the association of select HLA class II alleles and haplotypes with type 2 diabetes pathogenesis and/or response to therapy.

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